

Lipase-Producing Microorganisms for Use in Contaminated Fat and Oil Kitchen Wastewater Treatment

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ABSTRACT

Biodegradation of fats and oils in wastewater has a potential role in pollution control. Then, selection of efficient microorganisms for degrading fats and oils in laboratory level has been investigated by using single culture of *Acinetobacter* sp. (KUL8), *Bacillus* sp. (KUL39) and *Pseudomonas* sp. (KLB1) and mixed cultures of *Acinetobacter* sp. (KUL8) and *Pseudomonas* sp. (KLB1). The result showed that single culture of *Pseudomonas* sp. (KLB1) and mixed cultures of *Acinetobacter* sp. (KUL8), *Bacillus* sp. (KUL39) and *Pseudomonas* sp. (KLB1) could be reduced fat and oil content by 55.91% and 60.42%, respectively. These cultures were shown highest efficiency activities. Thus, these cultures were selected to study speed of shaking and inoculum size in wastewater treatment, which it was found that the efficiency of fat and COD degradation were highest reduced at 250 rpm of speed of shaking. Inoculum size at 1-2.5 % and 5 % of single culture and mixed cultures could remove fat and oil by 61.78-65.19 % and 60.42 %, respectively. And initial COD concentration at 4,000 mg/l has the highest efficiency 83.46% and 95.81% in fat and COD degradation, respectively.

The last experiment, *Pseudomonas* sp. (KLB1) was used in occasionally influent and effluent contaminated fat and oil kitchen wastewater treatment in reactor. The results showed that the highest efficiency of aerobic conditions were 50.02% and 0.76 U/ml, respectively in fat degradation and lipase activity after adding 8% of single culture at 22 days. After that lipase activity was reduced because influent and effluent of wastewater in reactor depended on activity of kitchen. Since dilution rates (D) of wastewater in reactor were higher than specific growth rate (μ), so microorganisms were washed out. Thus, the immobilization of microorganism cells on plastic balls has been used. The results showed that cells immobilization could increase efficiency of wastewater treatment in reactor.

Key words: bacteria; lipase; wastewater treatment

INTRODUCTION

Currently, Thailand has wastewater problem due to industrial development and increasing population. Domestic wastewater is rich in organic matter. It is mainly derived from kitchen wastes, which consisted of the remnants of vegetable oils and animal fats. Lipids constitute

about 20-30 % of this organic matter (Marty *et al.*, 1996). Lipids can cause great problems in wastewater systems, from the blockage of sewerage pipes to interfering with activated sludge oxygen transfer rate (Espinosa and Stephenson, 1996).

In general, treatment of lipid contaminated wastewater can be accomplished by simple physical operations such as skimming and adsorbing by

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using fabrics. On the other hand, chemical method is also used to be stabilize emulsion and followed by thermal or precipitation process. However, these chemical processes are costly. Thus, biological wastewater treatment is interested by lipase-producing microorganisms because lipid residues are converted to carbon dioxide, water and biomass (Lefebvre *et al.*, 1998).

The present study was aimed to optimize conditions and select strains of effective fat degradation in kitchen wastewater.

MATERIALS AND METHODS

Bacterial strains

The bacteria *Acinetobacter* sp. (KUL 8), *Bacillus* sp. (KUL 39) and *Pseudomonas* sp. (KLB 1) were obtained from Department of Biotechnology (Faculty of Agro-industrial, Kasetsart University, Thailand). Strains were maintained on nutrient agar (NA) slants at 4°C.

Growth media and cell cultivation

The bacterias were cultivated in 250 ml erlenmeyer flasks containing 50 ml of culture medium and incubated on rotary shaker at 250 rpm 35°C for 24 h. The composition of the medium was 0.1% (w/v) $(\text{NH}_4)_2\text{SO}_4$, 0.09% (w/v) K_2HPO_4 , 0.06% (w/v) KH_2PO_4 , 0.02% (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01% (w/v) yeast extract and 1% (v/v) olive oil.

Degradation of contaminated fat and oil kitchen wastewater

Treatment of contaminated fat and oil kitchen wastewater in laboratory level

The pH of wastewater was adjusted to 7.0 and blended for 5 min at maximum speed and used in the experiment.

Treatment of contaminated fat and oil kitchen wastewater in reactor

One % of *Pseudomonas* sp. was added in reactor, which consisted of 25 l wastewater.

Kitchen wastewater treatment used by immobilized cell

The immobilized cell and medium used for the enrichment of *Pseudomonas* sp. was rice bran instead of olive oil. To form a layer of biofilm on the surface of 120 plastic balls ($\varnothing$ 5 cm) in the 60 l reactors, that every day divided 20 balls, 1 l enrichment cultures of *Pseudomonas* sp. and 1 l fresh enrichment medium were introduced into the batch reactors. Aeration was pumped (0.24 vvm). After 10 days of incubation, the medium was added in reactors that contained 40 l wastewater in 60 l reactor.

Analytical procedure

The chemical oxygen demand (COD) was determined using the method described by Thonchai and Ausa (1992). The fats content was determined according to Standard Methods (APHA, 1998). The lipase activity was determined according to a modification of Horani (1996).

RESULTS AND DISCUSSION

Preliminary characterization of wastewater samples

The wastewater used in this study was taken from the grease trap of a kitchen wastewater of household. General properties of the wastewater sample were determined. The pH was 5.0–6.5, the BOD value was 120–2,400 mg/l, the COD values was 200–4,000 mg/l. The value for the fat/oil content was 15–20 g/l.

Effect of conditions and microorganisms on wastewater treatment in laboratory

Effect of single culture and mixed cultures

A comparison of the fats and oils degradation, the results showed that fat was highest degraded 55.91 % and 60.42 % in single culture of *Pseudomonas* sp. and mixed cultures of *Acinetobacter* sp. and *Pseudomonas* sp.,

respectively. For instance COD reduction showed that single culture of *Pseudomonas* sp. and mixed cultures could degrade for more than 90 % (Table 2). The differences in the fat degradation of the various cultures could depend on lipase system and physical properties of substrate (Wakelin and Forster, 1997; Tan and Gill, 1985). Thus, single culture of *Pseudomonas* sp. and mixed cultures were select for studied in the further experiment.

Shaking speed

In the experiment, speed of shaking was reduced from 250 rpm to 150 rpm because the wastewater treatment has been low aeration for reduced cost and difficult. The efficiency of fat

and COD reduction of single culture and mixed cultures shaking at 250 rpm were higher than shaking at 150 rpm (Table 3). Since, oxygen concentration was important for growth and could assist the action of enzyme. Corresponding to that obtained by Giuseppin (1984) found lipase production of *Rhizopus delemar* depended on oxygen concentration in medium because low oxygen concentrations have large effect on the growth of the fungus as well as *Geotrichum candidum* (Jacobsen *et al.*, 1989). Lipase has adsorption affinity with the oil droplets. Shape and size of the oil droplets play an important role on the rate of production of the enzyme (Tamerler and Keshavarz, 2000). Dalmau *et al.*, (1998) reported

Table 2 Degradation efficiency of lipase producing microorganisms in contaminated fat and oil kitchen wastewater treatment.

Microorganisms	Fat/Oil degradation (%)	COD decrease (%)
Control	9.03	58.54
<i>Acinetobacter</i> sp. (KUL 8)	17.13	77.10
<i>Bacillus</i> sp. (KUL39)	19.92	76.80
<i>Pseudomonas</i> sp. (KLB 1)	55.91	90.26
<i>Acinetobacter</i> sp. : <i>Pseudomonas</i> sp.	60.42	90.56

Table 3 Comparison speed of shaking to contaminated fat and oil kitchen wastewater treatment of single culture of *Pseudomonas* sp. and mixed cultures.

Microorganisms	Fat/Oil degraded (%)	COD degraded (%)
Shaking at 250 rpm		
Control	9.03	58.54
<i>Pseudomonas</i> sp.	55.91	90.26
<i>Acinetobacter</i> sp. : <i>Pseudomonas</i> sp.	60.42	90.56
Shaking at 150 rpm		
Control	14.53	36.78
<i>Pseudomonas</i> sp.	47.60	78.00
<i>Acinetobacter</i> sp. : <i>Pseudomonas</i> sp.	33.79	85.14

that lipase production from cultures of *Candida rugosa* increased with increasing droplet size.

Effect of inoculum size

The studies of inoculum size of single culture and mixed cultures were 1-2.5 % and 5 %, respectively which had the highest efficiency of fat degradation 61.78-65.19 % and 60.42 %, respectively. COD reduction were more than 85 % in all experiment (Table 4). These results showed that increasing inoculum size could not reduce fat degradation because microorganisms degraded fat to be intermediate, which inhibited lipase activity. Macris *et al.* (1996) studied triolein hydrolysis by

lipase that showed concentration of glycerol up to 150 mM inhibited triolein hydrolysis. The single culture of *Pseudomonas* sp. was selected for further experiment.

Effect of initial COD loading

Initial COD concentration 2,000, 3,000 and 4,000 mg/l in wastewater were used for study on fat and COD degradation by 1-2.5 % inoculum size of single cultures. Results obtained in Table 5 indicated that initial COD 4,000 mg/l had the highest efficiency 83.46 % and 95.81 % in fat and COD degradation, respectively. Reducing COD in wastewater is provided because microorganisms

Table 4 Effect of inoculum size in contaminated fat and oil kitchen wastewater treatment by single culture of *Pseudomonas* sp. and mixed cultures.

Inoculum size (%)	<i>Pseudomonas</i> sp.		<i>Acinetobacter</i> sp. <i>Pseudomonas</i> sp.	
	Fat/oil degraded (%)	COD degraded (%)	Fat/oil degraded (%)	COD degraded (%)
Control	7.93	67.50	7.93	67.50
1	61.78	92.01	23.46	89.34
2.5	65.19	87.52	26.72	85.14
Control	9.03	58.54	9.03	58.54
5	55.91	90.26	60.42	90.56
Control	4.51	66.21	4.51	66.21
10	51.32	89.03	57.14	88.03
20	47.42	87.64	45.28	89.14

Table 5 Effect of initial COD in contaminated fat and oil kitchen wastewater treatment by single cultures.

Initial COD (mg/l)	Fat/oil degraded (%)	COD degraded (%)
control	11.28	62.42
2,000	55.91	90.26
3,000	68.91	92.86
4,000	83.46	95.81

have nutrients, which enhance growth. Corresponding to the report of Rivas *et al.* (2000) that higher initial COD could be higher its removal rate.

Effective of microorganisms in wastewater treatment in reactor

1 %, 2 % and 4 % of *Pseudomonas* sp. inoculum were added in 0, 6 and 14 days, respectively. The results showed that fat and COD reduction were similar to control (no inoculum cultures) and could not detect lipase activity (Figure 1). Subsequently, 8 % adding cultures in 22 days were found the highest efficiency was 50.02 % and 0.76 μml in fat degradation and lipase activity in 36 days, respectively. COD degradation had the highest efficiency 85.00 % in 34 days. Characteristic of fat degradation and lipase activity was reduced because of the dilution rate (D) was higher than the specific growth rate (μ) so microorganisms were washed out. In Contrast

COD degradation, which was high because fat in wastewater into fatty acid and glycerol. Because of microorganisms used organic compound for growth, then COD degradation was high. Thus, the immobilization of microorganism cells had been used.

Immobilization cell in wastewater treatment

Immobilized cell on surface of 120 plastic balls was added in reactor. The first period found that fat content was rapid increasing and the highest at 4.10 g/l in 16 days, after that fat content was reduced 1.26 and 1.25 g/l in 18 and 20 day, respectively (Figure 2). Because of the first period, microorganisms were required acclimatization period and accumulation of fat along the inner wall. Then, microorganisms was cultivated enrich media must be required acclimatization. After that, lipase activity could detect in 4 to 20 days of experiment. Maximum lipase activity was the highest 1.07 μml in 16 days cultures.

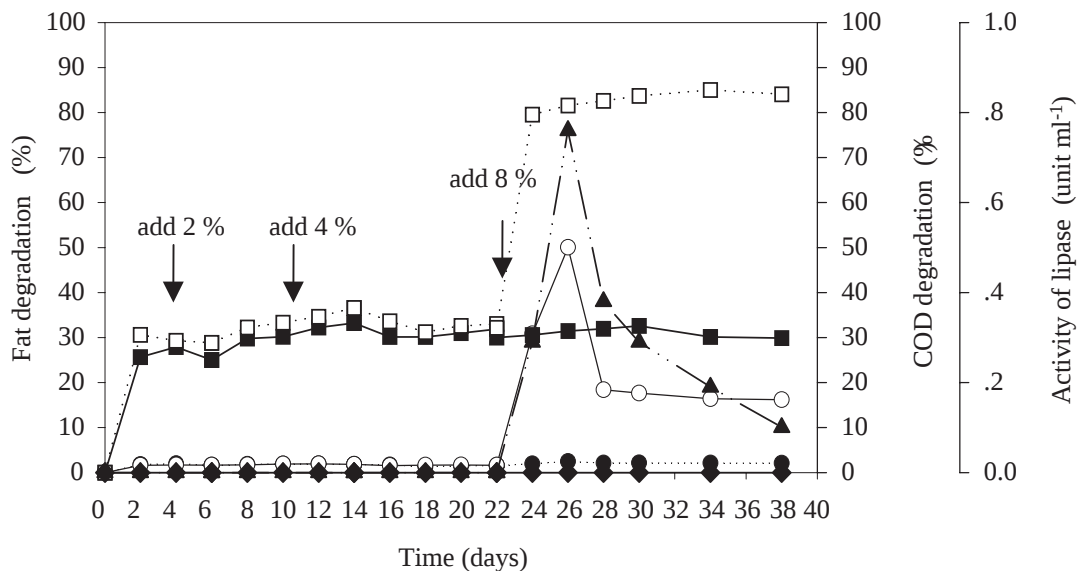


Figure 1 Effect of contaminated fat and oil kitchen wastewater treatment in aerobic conditions in reactor by *Pseudomonas* sp.

Fat degradation (● Control, ○ KLB1)
 COD degradation (■ Control, □ KLB1)
 Activity of lipase (▲ Control, △ KLB1)

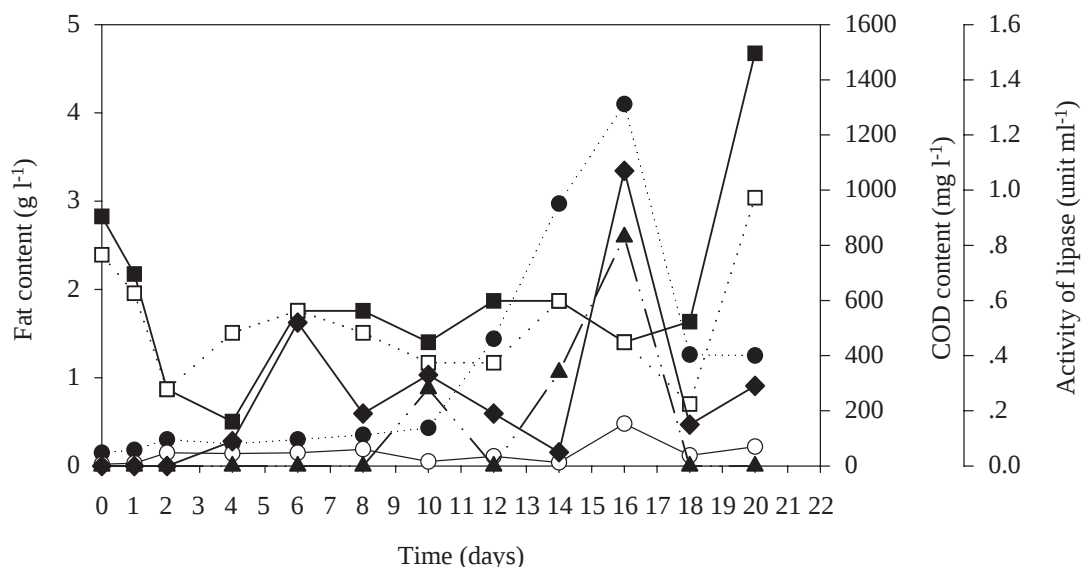


Figure 2 Effective of contaminated fat and oil kitchen wastewater treatment in aerobe conditions in reactor by immobilization cell.

Fat degradation (● Control, ○ KLB1)

COD degradation (■ Control, □ KLB1)

Activity of lipase (▲ Control, ◻ KLB1)

These results showed that microorganisms could grow in wastewater system of all experiment. Therefore, lipase activity in immobilization system was higher than suspension cell system. Microorganisms could grow on the support particles either in the form of biofilm or inside porous structure of balls.

CONCLUSION

Experimental results indicated that single culture of *Pseudomonas* sp. gave the highest efficiency of 83.46 % and 95.81 % in fat and COD degradation, respectively at the shaking rate of 250 rpm and having the initial COD of 4,000 mg/l. In study kitchen wastewater treatment in reactor found that 8 % of *Pseudomonas* sp. gave the highest efficiency of 50.02 % and 0.76 μ /ml in fat degradation and lipase activity, respectively. But the lipase activity was reduced because influent

and effluent of wastewater in reactor depended on activity of kitchen. Thus, the immobilization of microorganism cells on plastic balls has been used for increase efficiency of wastewater treatment in reactor.

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